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Invited Editorial Comment

Twins and Twinning

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Twins have fascinated humanity since the beginning of recorded history. This issue of the *American Journal of Medical Genetics* includes a series of papers on twins and twinning. It is a timely and important contribution because in the last few years there have been many new observations concerning twins.

The usefulness of twins in studying the genetic contribution to various traits was first suggested by Galton [1875]. He proposed that by comparing the concordance of a specific trait or disorder in monozygotic twins (MZ) with dizygotic twins (DZ) it would be possible to distinguish between environmental and heritable effects (nurture vs. nature). Galton's suggestion lead to the development of a body of knowledge which has been particularly useful in studying complex disorders [Bouchard et al., 1990; Phillips, 1993].

Recent technological advances in molecular and developmental genetics have uncovered a number of non-traditional mechanisms associated with discordance in MZ twins, e.g., mosaicism [Saul et al., 1990; Machin, 1995], imprinting, and uniparental disomy [Haskins-Olney et al., 1988]. These observations raise concerns about the premises upon which studies comparing MZ and DZ twins have been based. The study of the mechanisms leading to discordance in MZ twins and the role such mechanisms play in the human twinning process itself should provide new insights into early normal human development.

Prior to biochemical and molecular techniques, determination of zygosity was imprecise. Physical similarity was used for centuries. When it became clear that physical resemblance was not always an accurate discriminator, blood types and placentation [Benirschke, 1961] were used. However, blood types and serum proteins are not expected to always be discordant in DZ twins and placental membranes have occasionally been shown to be unreliable in establishing monozygosity [Bieber et al., 1981; Langlois et al., 1994, personal communication]. Furthermore, a single placenta with vascular connections between the twins is expected in at least 70% of liveborn MZ twins [van Dijk et al., 1994; Machin et al., 1995]. Thus, blood type, serum proteins, and even DNA studies from either twin will be expected to show admixture or chimerism even if concordance does exist.

The Weinberg method is used to give a rough mathematical estimation of the proportion of MZ and DZ twins in the population [Weinberg, 1902]. It is based on the relative number of like-sex and unlike-sex twin births assuming that the excess of like-sex twins are MZ twins. Using this method the overall birth incidence of twins (both MZ and DZ) is estimated to be 1 in 80 births or 1 in 40 individuals in North America. The Weinberg method may be useful for statistical purposes; however, for accurate twin research, zygosity must be established using placental membranes and/or DNA studies which are ideally done in tissues other than blood [Hill and Jeffreys, 1985; Jeffreys et al., 1985; Akane et al., 1991].

The number of DZ twins estimated by the Weinberg method varies among different ethnic groups (high among blacks, low among the Japanese) [Imaizumi, 1990] and is considered to be related to elevated gonadotropin levels leading to multiple ovulation [Nylander, 1981]. In the last decade the incidence of liveborn DZ twins has increased in association with the use of fertility drugs and in vitro fertilization techniques [Derom et al., 1991]. By contrast, the incidence of MZ twins had been thought to be constant worldwide at 1 MZ twin birth every 330 births [Nylander, 1975]; however, the incidence of MZ twins has been reported to increase with artificially induced ovulation and in vitro fertilization techniques [Edwards et al., 1986; Derom et al., 1987].

At a recent international conference on twins, data from several countries suggest that the incidence of all twin births is increasing [Bressers et al., 1987; Bryan, 1994] independent of fertility drugs. Czeizel et al. [1994] examined the effect of multivitamins in preventing congenital anomalies; he reported that vitamin supplementation prior to and in early pregnancy increased the occurrence of twin livebirths (both MZ and DZ). This observation suggests that the effect of vitamins in the prevention of congenital anomalies may maintain

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twin pregnancies, some of which would otherwise have been lost due to lethal congenital anomalies. Thus, the observed increase of liveborn twins in the general population may be related to better nutrition. The study of Czeizel et al. [1994] also shows an increase in recognizable miscarriages in the vitamin supplemented group, suggesting that even abnormal pregnancies may progress further into the pregnancy when the mother has nutritional supplementation.

With the development and use of ultrasonography in early human pregnancies, it has become clear that many more pregnancies start as twins than come to term delivery as twins. The term "vanishing twin" has been coined for those pregnancies which convert to singletons or are lost [Levi, 1976; Jeanty et al., 1981; Jauniaux et al., 1986]. Jauniaux et al. [1986] estimated that at least 70% of twin conceptions do not come to term as twins. A more recent report by Boklage [1990] estimated that multiple conceptions may constitute 12% of all pregnancies but that only 2% survive to term as twins. The incidence of twins among miscarriages is also high [Livingston and Poland, 1980]. The mechanism(s) by which one (or both) of the twins in a pregnancy abort or vanishes is not known. Some suggestions include postconceptional nondisjunction leading to chromosomal imbalance such as tetraploidy [Rudnicki et al., 1991], embryonic growth disorganization due to abnormal chromosomes [Livingstone and Poland, 1980], non-viable malformations in one or both twins, and disruptions such as vascular compromise of one or both twins [Landy et al., 1986]. It is not clear whether the "vanishing twin" phenomenon occurs primarily in MZ or DZ or equally in both types of twins.

An increase in most congenital anomalies is seen in one or both individuals of a twin pair [Källen, 1986]. In at least 10% of MZ twin pregnancies coming to birth, one or both twins has some type of congenital anomaly [Doyle et al., 1990]. These anomalies include malformations, disruptions, and deformations [Schinzel et al., 1979]. The true incidence of anomalies in MZ and DZ twins is hard to assess since many previous studies failed to determine zygosity correctly and some even assumed dizygosity if there was discordance because of the anomalies.

The cause of MZ twinning is not known. Familial MZ twinning (in which several relatives in a family have MZ twin pregnancies) is rare in humans [Harvey et al., 1977; Parisi et al., 1983; Shapiro et al., 1987a]. The cause of familial MZ twinning is thought to be related to a single gene possibly associated with inherited abnormalities of the zona pellucida or cell-to-cell connections which would allow cells to separate before implantation and placentation [Shapiro et al., 1987b]. Interestingly, no increase in congenital anomalies is apparent in familial MZ twinning. Abnormalities in the zona pellucida allowing early separation of cells, discordant X-inactivation in some female MZ twins [Burn et al., 1986; Goodship et al., 1984], or abnormalities in developmental clocks (i.e., time of fertilization, implantation, hatching, X-inactivation) [Boklage, 1987] have all been suggested for sporadic (non-familial) MZ twinning in humans.

Discordance has been observed frequently among MZ twins with disorders known to be genomically imprinted [Hall and Lopez-Rangel, 1995]. Discordance among MZ twins is also seen with mosaicism for chromosomal abnormalities, in many single gene (autosomal dominant and X-linked) disorders and with cytoplasmic inheritance. Based on these observations we have postulated that the development of a discordant cell line (by any mechanism including chromosomal, single gene mutation, mitochondrial mutation, uniparental disomy, somatic crossing over, X-inactivation, imprinting, etc.) early in development may be a cause for human MZ twinning (often without obvious physical discordance). In order to demonstrate discordance between MZ twins, it is important to remember that 70% of MZ twins who come to term have shared their circulation. Thus, examining white blood cell DNA may not demonstrate cytogenetic, cytoplasmic, and/or molecular discordance even though it exists. Fibroblasts (skin biopsy) is a much better tissue to look for discordance when studying MZ twins.

The study of twins and the twinning process will greatly benefit from the use of the new genetic technologies. Proper use of DNA techniques can provide unequivocal differentiation between MZ and DZ twins as well as uncovering a number of different types of genetic discordance. The availability and accuracy of these new techniques will allow new studies (or the repetition of old studies) involving twins to be conducted with the advantage of properly established zygosity. Hopefully subgrouping of twins from now on will not only be based on accurately diagnosed zygosity, but also on a description of placentation, placental connections, and molecularly defined discordance. There is much work to be done and much to be learned!

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